Influences of the *Populus deltoids* seedlings treated with exogenous methyl jasmonate on the growth and development of *Lymantria dispar* larvae

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Abstract: *Lyantria dispar* larvae were fed with the leaves of *Populus deltoids* seedlings exposed to methyl jasmonate (MeJA) for 24 h. The growth and development of the larvae were investigated, and phenolics contents in treated leaves including pyrocatechol, caffeic acid, coumarin, ferulic acid and benzoic acid were also surveyed by high-pressure liquid chromatography (HPLC). Results indicated that approximate digestibility, efficiency of conversion of ingested food, efficiency of conversion of digested food, and weight of the larvae were inhibited obviously, especially from the sixth day, which may result from the increase of total phenolics contents in treated leaves. This result provides strong supports for MeJA acting as the airborne signal molecule between woody plants.

Keywords: Interplant communication; Defense response in *Populus deltoids* plants; Exogenous MeJA; Growth and development of *lymantria dispar*; Phenolics contents
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Introduction

The interplant signal transduction has been hypothesized for many years (Baldwin and Schultz 1983; Rhoades 1983). It has been found that the resistance to insects is induced not only in damaged plants but also in the neighboring undamaged individuals. Recent studies have demonstrated that when plants are attacked by insects, neighboring intact plants can be affected by volatile signals and can, as do highly infected plants, become repellent to incoming herbivores and increasingly attractive to foraging predators and parasitic insects (Pickett 2003). Arimura *et al*. (2000) reported that several volatiles, including β-ocimene, 4,8-dimethylnona-1,3,7-triene (DMNT), and 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), act as the interplant signals that can induce the expression of pathogen-related (PR) genes and other defense genes in the undamaged lima bean leaves. Methyl jasmonate (MeJA) is also considered to be a candidate for interplant communication and may induce resistance to insects. MeJA, as a volatile derived product of JA, is synthesized in plant via the octadecanoid biosynthesis pathway (Kessler and Baldwin 2002; Seo *et al*. 2001). Briefly, linolenic acid from the membrane is oxygenated by lipoxygenase (LOX), and then converted to 12-oxo-phytodienoic acid (12-oxo-PDA) by allene oxide synthase (AOS) and allene oxide cyclase (AOC). Jasmonic acid is synthesized from the 12-oxo-PDA through reduction and three steps of β-oxidation. Cellular organelles such as plastids or peroxisomes are regarded as the primary sites of JA biosynthesis. JA is then catabolized further by jasmonic acid carboxyl methyltransferase (JMT) to form its volatile counterpart MeJA (Seo *et al*. 2001; Ma *et al*. 2001).

Many studies showed that MeJA is emitted from plants after attack by insects or pathogens (Rakwal and Komatsu 2000; Schweizer *et al*. 1997; Kessler and Baldwin 2001), and much evidence suggests that MeJA may induce the defense response in intact plants. Treatment of barley *(Hordeum vulgare* L.cv.Golden Promise) seedlings with MeJA leads to a significant decrease in powdery mildew (*Blumeria graminis* f.sp.hordei) infection, as well as great increasing activity of the defense-related enzymes, such as phenylalanine ammonia lyase (PAL) and peroxidase (POD) in both treated leaves and systemic leaves (Walters *et al.* 2002). The exogenous MeJA can induce the biosynthesis of proteinase inhibitors (PIs) in the intact leaves of tomato, which is similar to the results of insects attack and mechanical damage (Farmer *et al*. 1992), and it also leads to the systemic increase in activity of polyphenol oxidase (PPO) (Thaler 1999). The potato plants applied by MeJA exhibit a high resistance to pathogens via the biosynthesis of some inducible defense proteins and phytoalexins (Cohen *et al*. 1993; Wang and Jiang 2002). The evidence above provides a strong support for MeJA as interplant signal between herbage plants, but little is known about its function between woody plants.

In our preliminary studies, after exposure to MeJA, the abscisic acid (ABA) content in poplar (*P. simonii*× *P. pyramibalis* c.v) seedlings is found to reach a high level (Yang *et al*. 2003). In order to confirm the effect of MeJA, as an interplant alarming signal between woody plants, in this work, *Lymantria dispar* larvae were fed with the leaves exposed to MeJA, then the growth and development of the insects was investigated, and the phenolics contents in treated leaves were also measured.

Materials and methods

Plant materials

The poplar (*Populus deltoides*) seedlings, provided by China Academy of Forestry, were transplanted to plastic pots in March watered every day, and irrigated with nutritive solution (Hoagland) every two weeks. When the plants were about 1.3 m

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high, they were used in the experiments.

Animal materials

Lymantria dispar larvae were used in this study. The larvae fed on the treated and control leaves separately were reared in a growth chamber maintained under 16 h light and 8 h darkness at (25±0.5)°C and 70%–75% humidity. When the larvae grew to 0.300 g, the indexes of growth and development were investigated.

MeJA treatments

The experiments were conducted in a close chamber built with the plastic membrane which gave out few volatile. MeJA obtained from Sigma-Aldrich Company was dissolved in ethanol to 1.00 M. An intact seedling was put into the chamber with a piece of absorbent cotton absorbing MeJA solution on an evaporating dish, leading to the final concentration 0.8 μM. The absorbent cotton was placed about 30 cm from the seedling. After 24 h, leaves of the plant were fed to larvae of the *Lymantria dispar*. And at 4 h, 1 d, 6 d and 12 d, the leaves were harvested to survey the phenolics contents. The control plant was fumigated with ethanol only under the same condition.

Indexes of the growth and development

The following equation was used to describe the food consumption of the larvae:

$$\gamma = (A - B)(1 + E)$$

where, γ is the food consumption of the larvae, *A* the weight of leaves before being fed, *B* the weight of leaves after being fed the next day, and *E* is the loss rate of water in leaves (%).

The food consumption and utilization of the larvae were evaluated according to Waldbauer Equation:

Approximate digestibility (AD)=
(feeding amount-excretion)/ feeding amount
Efficiency of conversion of ingested food (ECI)=
weight / feeding amount
Efficiency of conversion of digested food (ECD)=
weight / (feeding amount-excretion)

Determination of phenolics contents

The phenolics contents were determined by high-pressure liquid chromatography (HPLC). HPLC system of phenolics assay was composed of Kromasil C18 reverse-phase column which was μ-Bondapak Phenyl with 0.4 cm × 30 cm, the mobile phase which contained 35% methanol, 3% acetonitrile, 62% H_2O, regulating pH value to 3.0 with phosphoric at a flow rate of 0.7 $mL \cdot min^{-1}$, and the detector which was UV254 nm × 0.1 AUFS.

Results and analysis

Influences of poplar seedlings exposed to MeJA on food consumption of larvae

The food consumption of larvae fed on control leaves increased gradually, and at the sixth day the food consumption of larvae took on a faster rise, which implied that from the sixth day the development of larvae entered a rapid growth period (Fig. 1). The food consumption of larvae fed on treated leaves exhibited the similar trend. Before the sixth day the food consumption of the treated larvae was almost as same as that of the control, but from the sixth day that of the treated larvae increased rapidly than that of the control, which meant the larvae ate more treated leaves.

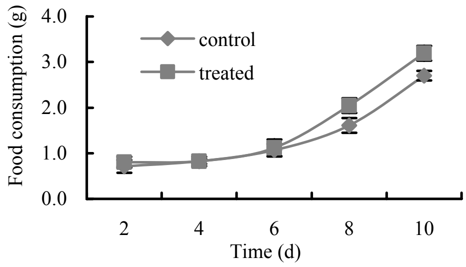

Fig. 1 Food consumption of larvae

Influences of poplar seedlings exposed to MeJA on nutritional metabolism of larvae

MeJA treatment on leaves influenced the digestibility of larvae (Fig. 2). It was found that the digestibilities of the treated and the control both rose regularly, which indicated a gradual increase of the assimilation in the larvae. The increasing rate of the digestibility of treated larvae was slower than that of the control obviously. Although at the beginning the digestibility of the treated larvae was higher, from the forth day that of the treated larvae became lower and lower.

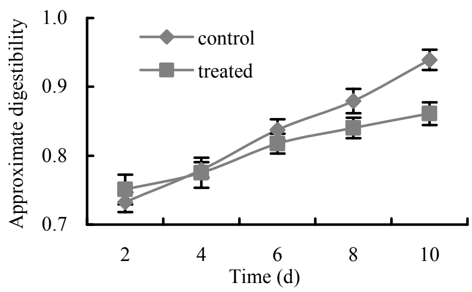

Fig.2 Approximate digestibility (AD) of the larvae

Fig. 3 and Fig. 4 depict the food conversion rate of digested food and efficiency of utilization of ingested food of the larvae, respectively. The curves of the treated and the control showed a similar trend: during the six days of beginning, ECD and ECI of the larvae increased rapidly, and then contrarily decreased gradually with the larvae growth, but ECD and ECI of the larvae fed on leaves treated by MeJA were much lower compared with those of the control.

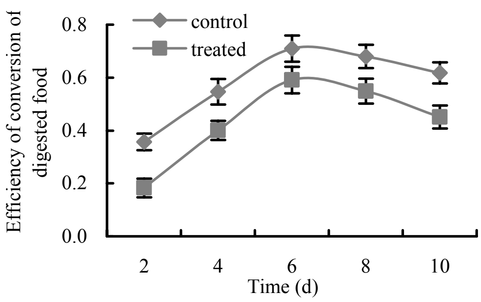

Fig. 3 Efficiency of conversion of digested food (ECD) in larvae

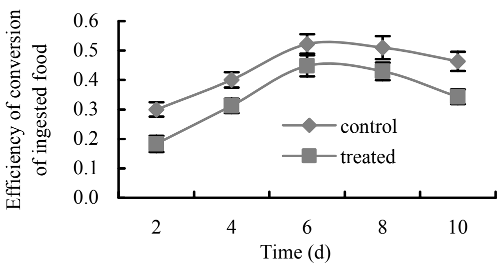

Fig. 4 Efficiency of conversion of ingested food (ECI) in larvae

The weight of larvae fed on the treated leaves increased slowly (Fig. 5). From the second day to the tenth day, the weight of the control larva increased gradually and stably, but the weight of the treated larvae showed a slower increase from the sixth day. Correspondingly the food consumption, AD, ECD and ECI of the treated larvae also showed obvious change at the sixth day.

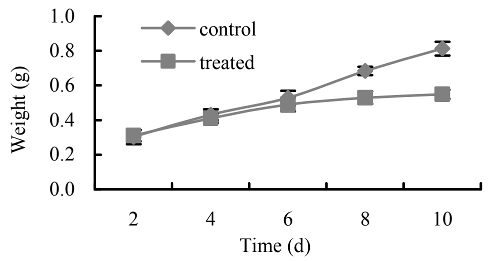

Fig. 5 Weight change of larvae

Phenolics contents in leaves exposed to MeJA

Phenolics, including pyrocatechol, caffeic acid, coumarin, ferulic acid, benzoic acid, were measured by HPLC (Fig.6). All of the phenolics contents increased after one day of treatments, and except pyrocatechol, other four phenolics contents reached the peak at the sixth day, then decreased at the twelfth day. Though pyrocatechol content was lower than that of the control, the total phenolics content greatly rose in the treated leaves.

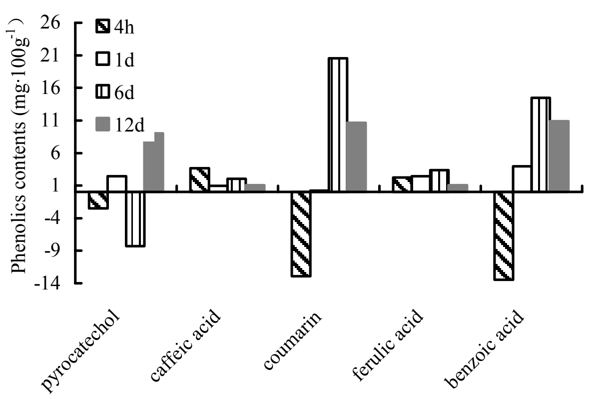

Fig. 6 Phenolics contents in leaves exposed to MeJA

Conclusion and suggestion

The food consumption, approximate digestibility (AD), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), and weigh of the *Lymantria dispar* larvae were affected by feeding on the poplar leaves treated by MeJA. Of those, except food consumption other four indexes of the treated larvae are lower than those of the control. The phenolics contents in the leaves after exposure to MeJA for one day increases significantly. Phenolics are contributed to decrease the nutrient contents and the edibility of leaves. Many studies also demonstrated that when herbage plants are fumigated by MeJA, various defensive pathways are stimulated, and many defense genes are activated leading to biosynthesis of diverse secondary metabolites and proteins against insects, such as alkaloids, terpenoids, phenolics (Keinänen *et al.* 2001), PIs (Bolter and Jongsma 1995) and PPO (Constabel and Ryan 1998). Thus the larvae must take more leaves treated by MeJA compared with the control especially from the sixth day after larvae entering the fast growth period. But due to the toxicity of secondary metabolites, AD, ECI, ECD and weight of the treated larvae are lower than those of the control. It is also observed that from the sixth day the growth and development of larvae fed with treated leaves is obviously inhibited in this experiment. Therefore the sixth day is the critical point in the growth and development of larvae. The effect of inhibition is more and more obvious in the middle and latter periods of the development of the larvae, which implies a great negative effect on their filial generation.

From above it is concluded that MeJA treatments may enhance resistance of intact poplar seedlings to *lymantria dispar* larvae, the total phenolics contents increase significantly in *Populus deltoides* leaves after exposure to MeJA in this study. This result provides a strong support for MeJA functioning as an airborne interplant signal transferring wound information from infected plant to neighboring intact plant to elicit defense response in woody plants. The further research needs to be conducted to investigate how MeJA combines with the receptor on the membrane of plant cells to trigger the expression of defense genes and the influences of the induced resistance on the filial generation, which will ground the new-style and effective pest management.

References

Arimura, G., Ozawa, R., Shimoda, T., Nishioka, T., Boland, W., Takabayashi, J. 2000. Herbivory induced volatiles elicit defence genes in lima bean leaves [J]. Nature, **406**: 512–515.

Baldwin, I.T., Schultz, J.C. 1983. Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants [J]. Science, **221**: 277–278.

Bolter, C.J., Jongsma, M.A. 1995. Colorado potato beetles (Leptinotarsa decemlineata) adapt to proteinase inhibitors induced in potato leaves by methyl jasmonate [J]. Journal of Insect Physiology, **41**: 1071–1078.

Cohen, Y., Gisi, U., Niderman, T. 1993. Local and systemic protection against phytophthora infestans induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester [J]. .Phytopathol., **83**: 1054–1062.

Constabel, C.P., Ryan, C.A. 1998. A survey of wound- and methyl jasmonate-induced leaf polyphenol oxidase in crop plants [J]. Phytochemistry, **47**: 507–511.

Farmer, E.E., Johnson, R.R., Ryan, C.A. 1992. Regulation of expression of proteinase inhibitor genes by methyl jasmonate and jasmonic acid [J]. Plant Physiol, **98**: 995–1002.

Keinänen, M., Oldham, N.J., and Baldwin, I.T. 2001. Rapid HPLC Screening of Jasmonate-Induced Increases in Tobacco Alkaloids, Phenolics, and Diterpene Glycosides in Nicotiana attenuate [J]. J. Agric. Food Chem., **49**: 3553–3558.

Kessler, A., Baldwin, I.T. 2001. Defensive function of herbivore-induced plant

volatile emissions in nature [J]. Science, **291**: 2141–2144.

Kessler, A., Baldwin, I.T. 2002. Plant responses to insect herbivory: The Emerging Molecular Analysis [J]. Annu Rev Plant Biol, **53**: 299–328.

Ma Chongjian, Liu Jun, Xie Conghua. 2001. Functions of Jasmonates and Defense of Stress [J]. Journal of Huazhong Agricultural University, **20**: 603–608. (in Chinese)

Pickett, J.A., Rasmussen, H.B., Woodcock, C.M., Matthes, M., Napier, J.A. 2003. Plant stress signalling: understanding and exploiting plant–plant interactions [J]. Biochem Soc Trans, **31**: 123–127.

Rakwal, R, Komatsu, S. 2000. Role of jasmonate in the rice (*royza sativa* L.) self-defense mechanism using proteome analysis [J]. Electrophoresis, **21**: 2492–2500.

Hedin, P.A. 1983. Plant resistance to insects [M]. Washington: American Chemical Society, p55–68.

Schweizer. P., Buchala, A., Silverman, P., Seskar, M., Raskin, I., Metraux, J.P. 1997. Jasmonate inducible genes are activated in rice by pathogen attack without a concomitant increase in endogenous jasmonic acid levels [J]. Plant Physiol., **114**: 79–88.

Seo, H.S., Song, J.T., Cheong, J.J., Lee, Y.H., Lee, Y.W., Hwang, I., Lee, J.S., Choi, Y.D. 2001. Jasmonic acid carboxyl methyltransferase:A key enzyme for jasmonate-regulated plant responses [J]. PNAS, **98**: 4788–4793.

Thaler, J.S. 1999. Jasmonate-induced plant defences cause increased parasitism of herbivores [J]. Nature, **399**: 686–688.

Yang Di, Li Qing, Hu Zenghui, Zhao Ling, Shen Yingbai, Chen Huajun. 2003. Content changes of abscisic acid and jasmonic acid in damaged and neighboring healthy ashleaf maple seedlings [J]. Journal of Beijing Forestry University, **25**: 35–38. (in Chinese)

Walters, D., Cowley, T., Mitchell, A. 2002. Methyl jasmonate alters polyamine metabolism and induces systemic protection against powdery mildew infection in barley seedlings [J]. Journal of Experimental Botany, **53**: 747–756.

Wang Niyan, Jiang Dean. 2002. The role of jasmonic acid and methyl jasmonate in plant induced disease resistance [J]. Plant Physiology Communications, **38**: 279–284. (in Chinese)